

MUTANT APICAL DOMINANCE GENE IN *EUSTOMA*

Related Application Information

5 *INS A2* This application is a continuation-in-part of U.S. application number 60/106,510, filed on October 30, 1998.

Field of the Invention

10 The present invention relates to an apical dominance allele. In *Eustoma*, this allele results in plants which exhibit a reduction in apical dominance and hence, an increase in basal and total branching. The present invention also relates to *Eustoma* seed, a *Eustoma* plant, a *Eustoma* variety and a *Eustoma* hybrid which contain this allele. In addition, the present invention also relates to methods for transferring this allele in a *Eustoma* plant to other *Eustoma* varieties and species and is useful for producing novel types and varieties of
15 *Eustoma* which exhibit a reduction in apical dominance and hence, an increase in basal and total branching.

Background of the Invention

20 *Eustoma* (*Eustoma grandiflorum*), commonly known as prairie gentian or Texas Bluebell, originated as a common North American wildflower. Originally botanists categorized *Eustoma grandiflorum* in the *Lisianthus* genus. Although the classification was changed, it is still referred to commonly as lisianthus. *Eustoma* is used as an annual herbaceous ornamental crop introduced into cultivation as a flowering-pot plant, bedding plant and a cut flower. It is available in a range of colors including white, light yellow, pink,
25 rose, lilac and deep purple-blue to a variety of bicolours. Flowers can be single or double.

At present, high light areas are needed to produce a well-branched pot *Eustoma*. It would be desirable to produce a *Eustoma* having increased branching to broaden production into lower light areas. In addition, increased branching in a *Eustoma* would offer better
30 show in the garden through improved habit and associated increased flower production.

Such a trait incorporated into cut flower *Eustoma* could increase cut stem yield per plant.

Summary of the Invention

5 The present invention relates to a *Eustoma* plant having reduced apical dominance. The *Eustoma* plant of the present invention contains an allele for reduced apical dominance. Additionally, the *Eustoma* plant of the present invention has a pedigree which includes the plant 752 or derivatives thereof, such as, but not limited to, 1389-1-1-9-4-3, 3000-1-2-1-1-1, 3107-1-1-3, 3130-1-3-1-1, 3030-1-4-2-5 or 3000-2-3-5-ml-2-2. The present invention also relates to seed, pollen, ovules, tissue culture and cuttings of a *Eustoma* plant having
10 reduced apical dominance.

The present invention further relates to *Eustoma* seed containing an allele for reduced apical dominance. The *Eustoma* seed of the present invention also has a pedigree which includes the plant 752 or derivatives thereof, such as, but not limited to, 1389-1-1-9-4-3, 3000-1-2-1-1-1, 3107-1-1-3, 3130-1-3-1-1, 3030-1-4-2-5 or 3000-2-3-5-ml-2-2. The
15 present invention also relates to a plant produced by growing the above-described seed.

The present invention also relates to a reduced apical dominance allele which when expressed in a *Eustoma* plant produces a reduced apical dominant phenotype in said plant.
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The present invention further relates to a method for producing F₁ hybrid *Eustoma* seed. The method involves crossing a first parent *Eustoma* plant with a second parent *Eustoma* plant and harvesting the resultant F₁ hybrid *Eustoma* seed. The first and second parent *Eustoma* plants used in this method exhibit reduced apical dominance. The present
25 invention also relates to a first generation (F₁) hybrid plant which is produced by growing hybrid *Eustoma* seed produced by the above-described method.

The present invention also relates to a process for identifying a *Eustoma* plant, the genome of which contains an allele for reduced apical dominance. This process involves

crossing a *Eustoma* plant to be tested with a *Eustoma* plant, the genome of which contains an allele for reduced apical dominance, recovering the resulting F₁ hybrid *Eustoma* seed, planting the F₁ hybrid *Eustoma* seed and regenerating into plants and selecting *Eustoma* plants, the genome of which contains the allele for reduced apical dominance, and which exhibit a reduced apical dominance phenotype.

The present invention also relates to a process of obtaining hybrid *Eustoma* seed, the genome of which contains an allele for reduced apical dominance. The process involves crossing a *Eustoma* plant obtained using the hereinbefore described process with a *Eustoma* plant, the genome of which contains an allele for reduced apical dominance and recovering the resulting hybrid *Eustoma* seed, the genome of which contains an allele for reduced apical dominance.

The present invention also relates to a process for obtaining a hybrid *Eustoma* plant the genome of which contains an allele for reduced apical dominance. The process involves crossing a *Eustoma* plant obtained using the hereinbefore described process with a *Eustoma* plant, the genome of which contains an allele for reduced apical dominance, recovering the resulting hybrid *Eustoma* seed, the genome of which contains an allele for reduced apical dominance and planting the hybrid *Eustoma* seed and regenerating into plants, the genome of which contains the allele for reduced apical dominance.

Finally, the present invention relates to viable *Eustoma* seeds and plants and succeeding generations thereof grown from seeds deposited under ATCC Accession number 203392 and *Eustoma* seeds and plants to which the allele for reduced apical dominance is transferred from the deposited seeds in succeeding generations thereof.

Brief Description of the Figures

Figure 1A shows the *Eustoma* cultivars of the present invention which contain the reduced apical dominance allele and which exhibit an increase in basal and total branching.

Figure 1B show *Eustoma* plants which do not contain the reduced apical dominance allele. These plants do not exhibit an increase in basal and total branching.

Figure 2 shows the RAD *Eustoma* hybrid 3087 (labeled as “A” in the photograph) compared to commercial hybrids “Blue Lisa” (labeled as “B” in the photograph) and “Florida Blue” (labeled as “C” in the photograph).

Figure 3 shows the RAD *Eustoma* hybrids 3214 (labeled as “A” in the photograph), 3213 (labeled as “B” in the photograph) and 3211 (labeled as “C” in the photograph) compared to the commercial hybrid “White Lisa” (labeled as “D” in the photograph).

Figure 4 shows the RAD *Eustoma* hybrid 3215 (labeled as “A” in the photograph) compared to the commercial hybrids “Pink Lisa”(labeled as “B” in the photograph) and “Florida Pink” (labeled as “C” in the photograph).

Detailed Description of the Invention

The *Eustoma* cultivars of the present invention exhibit a substantial reduction in apical dominance. A transferrable allele which results in reduced apical dominance and hence, increased basal and total branching, has been discovered and incorporated into other genetic backgrounds. As used herein, the term “reduced apical dominance” (hereinafter referred to as “RAD”), means that apical dominance is reduced such that plants expressing this trait have increased basal branching when compared with commercial hybrids. As used herein, the term “basal branching” refers to the branches arising from the cotyledonary node below the first true leaves. The RAD hybrids also demonstrate high levels of total branching. As also used herein, the term “total branching” refers to shoots arising from the basal branches and from the main stem above the first true leaves. Additionally, multiple branches per leaf axil are present under high light conditions. As used herein, the term “high light conditions” means that the amount of light present per 24 hour day is from about 20 to about 30 mol•m⁻²•day⁻¹.

Eustoma cultivars which do not contain the allele described herein branch poorly when grown under low light conditions. As used herein, the term "low light conditions" means that the amount of light present per 24 hour day is from about 5 to about 6 mol• m⁻²• day⁻¹. In contrast, *Eustoma* cultivars containing the RAD allele described herein branch under low light conditions.

The RAD allele was discovered as a result of breeding and research efforts which were conducted at Linda Vista, Costa Rica. In 1987, a cross was made using a *Eustoma* line received from the United States Department of Agriculture. This *Eustoma* line did not exhibit any reduced apical dominance and was disease susceptible. This line was crossed with a second, proprietary *Eustoma* line which did not exhibit any reduced apical dominance. Seed from this cross was collected and planted. In the resulting F₁ population, none of the resulting plants exhibited any reduced apical dominance. The plants of the F₁ population were then self-fertilized. The seed from this self-fertilization was collected and planted. In the resulting segregating F₂ population, a single mutant inbred *Eustoma* line, designated 752, was discovered which exhibited reduced apical dominance. The remaining plants in this segregating population did not exhibit any reduced apical dominance. Proprietary inbred *Eustoma* line 752 exhibits the RAD trait, is only about 12 cm in height and is susceptible to many types of diseases. A breeding program was begun using proprietary inbred *Eustoma* line 752 to introduce the RAD trait into other *Eustoma* genetic backgrounds. Specifically, this proprietary inbred line was crossed with other *Eustoma* lines containing commercially desirable traits, such as improved disease resistance. It may also be transferred to taller *Eustoma* plants to increase the cut stem yield, or to create vigorous branched garden plants. Additionally, polyploid *Eustoma* plants can be created through various methods, including application of colchicine, protoplast fusion, and other well known techniques.

Based on their breeding and research efforts as described herein, and while not wishing to be bound by any theory, the inventors believe that the RAD trait described herein

is controlled by a single recessive gene. However, it is contemplated that it is possible that in certain genetic backgrounds that the RAD trait may have a more complex inheritance.

5 The *Eustoma* cultivars of the present invention are genetically stable and can be stably reproduced by means of asexual or sexual propagation. Additionally, the RAD allele which conveys this reduced apical dominance can and has been, bred into diverse *Eustoma* genetic backgrounds.

10 The *Eustoma* cultivars of the present invention maintain functional male and female organs, thus making the incorporation/introgression of the RAD trait into the genetic backgrounds of other *Eustoma* cultivars possible. Introgression of RAD gene into a desired genetic background can be performed by applying pollen from one plant to the exposed stigma of the flower of another plant. The trait for RAD may be incorporated into cultivars with many different flower colors, including cultivars with bicolor flowers, as well
15 as into cultivars having single, double and semi-double flowers. Well-known *Eustoma* plants having such characteristics are readily available.

After obtaining *Eustoma* plants having a desired blend of traits, these plants can be propagated using standard methods known in the art. Specifically, it is expected that any
20 selected RAD *Eustoma* can be produced commercially through asexual propagation. All RAD *Eustoma* tested thus far have been found to be stable through asexual propagation. Cuttings for asexual propagation can be taken at any time of the year and no special soil mixtures are required. Hormones, such as, indole-3-butyric acid (referred to as "IBA") or naphthaleneacetic acid (referred to as "NAA") may be used as needed.

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Additionally, it has been demonstrated that the *Eustoma* cultivars of the present invention can be produced as progeny from sexual crosses for the purposes of selling seed. Methods for the storage of such seed are well known in the art.

Since, as described hereinbefore, the RAD trait appears to be controlled by a single recessive gene, it is possible to determine whether a particular plant carries the RAD gene by performing an allelism test. This test is performed by crossing the test plant with a plant exhibiting the RAD trait. If the product of the cross also has the RAD trait, then the test plant carries the RAD gene. On the other hand, if none of the hybrid progeny exhibit a substantial reduction in apical dominance, then the branching of the test plant is not due to the RAD gene.

By way of example, and not of limitation, examples of the present invention shall now be given.

EXAMPLE 1: Reduced Apical Dominance in Blue Hybrid *Eustoma* 3087

Eustoma hybrid 3087 is a cross between two reduced apical dominant lines, 1389-1-1-9-4-3 (hereinafter, the "1389 line") x 3000-1-2-1-1-1 (hereinafter, the "3000" line). The 1389 line has a height of 30 cm and was derived from a cross between *Eustoma* line, 5-2-5-4, and *Eustoma* line 752. Line 5-2-5-4 has flowers which are ivory in color, a height of about 45 cm and is disease resistant. Seed from this cross was collected and planted. An individual F₁ plant was selected and self-pollinated, and the resulting seed was collected and planted. In the resulting F₂ population, a plant containing the reduced apical dominant character was selected. This selected plant, referred to as 1389-1-1, was then self-fertilized for three (3) generations.

The 3000 line was derived from a cross between *Eustoma* line 752 and "White Lisa" (developed by and commercially available from Pan American Seed Company, 622 Town Road, West Chicago, IL 60185). "White Lisa" is about 16 cm in height and was derived from a cross between 31-14-1-2-9 which has ivory flowers and is about 16 cm in height and 13-25-2-1-9 which has ivory flowers and is about 16 cm in height. Seed from this cross was collected and planted. An individual F₁ plant was selected and self-pollinated, and the resulting seed was collected and planted. In the resulting F₂ population, a plant containing

the reduced apical dominant character was selected. This selected plant, referred to as 3000-1-2, was then self-fertilized for three (3) generations.

5 *Eustoma* hybrid 3087 contains the RAD allele and is a multiple branched semi-dwarf *Eustoma* having a height of about 30 cm. The branching of this hybrid starts when the hybrid is in the plug stage. The flower is a campanulate type having a diameter of about 5.5 cm and is deep blue in color (2617C in the *Pantone Color Selector 1000 Coated 5th Printing* 1993-1994) with shading to lighter blue in the center. The leaves of this *Eustoma* are 4 x 3 cm (length x width) and are medium green (POLT-C in the *Pantone Color Selector 1000* 10 *Coated 5th Printing* 1993-1994) in color. Additionally, *Eustoma* hybrid 3087 exhibits some resistance to soil borne diseases.

Eustoma hybrid 3087 can be used in sexual crossings to introgress the RAD gene into the genetic backgrounds of other *Eustoma* plants having commercially desirable 15 characteristics in order to create new plants exhibiting the RAD trait.

EXAMPLE 2: Reduced Apical Dominance in Light Sky Blue *Eustoma* Hybrid 3212.

Eustoma hybrid 3212 is a cross between two reduced apical dominant lines, 3107-1-1-3 (hereinafter, the “3107 line”) x 3130-1-3-1-1 (hereinafter, the “3130 line”). The 3107 20 line has a height of 16 cm and was derived from a cross between *Eustoma* line, 3003-1-1-1-3 and *Eustoma* line 3026-2-3. 3003-1-1-1-3 has flowers which are pink in color, a height of about 16 cm and was derived from a cross between 752 x JP29. Seed from this cross was collected and planted. An individual F₁ plant was selected, and self-pollinated and the resulting seed was collected and planted. In the resulting F₂ population, a plant containing 25 the reduced apical dominant character was selected. This selected plant, referred to as 3107-1-1, was then self-fertilized for one (1) generation.

The 3130 line was derived from a cross between *Eustoma* line 3179-1-2-4 and 3026-3-2. 3026 is about 16 cm in height and was derived from a cross between *Eustoma*

line 3013-1 which has sky blue flowers and is about 19 cm in height and *Eustoma* line 3000-2-3-2 which has blue flowers and is about 16 cm in height. The 3000 line was derived from a cross between 752 x "White Lisa". Seed from this cross was collected and planted. An individual F₁ plant was selected and self-pollinated, and the resulting seed was collected and planted. In the resulting F₂ population, a plant containing the reduced apical dominant character was selected. This selected plant, referred to as *Eustoma* line 3130-1-3, was then self-fertilized for two (2) generations.

Eustoma hybrid 3212 contains the RAD allele and is a multiple branched dwarf *Eustoma* having a height of about 16 cm. The branching of this hybrid starts when the hybrid is in the plug stage. The flower is a campanulate type having a diameter of about 5.25 cm and is light sky blue in color (ECOO-C, 2706C *Pantone Process Color Imaging Guide 1000*, 1992).

The leaves of this *Eustoma* are 6 x 3.5 cm (length x width) and are medium green.

Eustoma hybrid 3212 can be used in sexual crossings to introgress the RAD gene into the genetic backgrounds of other *Eustoma* plants having commercially desirable characteristics in order to create new plants exhibiting the RAD trait.

EXAMPLE 3: Reduced Apical Dominance in Ivory *Eustoma* Hybrid 3213

Eustoma hybrid 3213 is a cross between two reduced apical dominant lines, 3030-1-4-2-5 (hereinafter, the "3030 line") x 3000-2-3-5-ml-2-2 (hereinafter, the "3000" line). The 3030 line has a height of 19 cm and was derived from a cross between *Eustoma* line 3000-3-1 and *Eustoma* line 13539-3. 3000-3-1 (which is a cross between 752 x "White Lisa") has flowers which are ivory in color, a height of about 16 cm and is disease resistant. Seed from this cross was collected and planted. An individual F₁ plant was selected and self-pollinated, and the resulting seed was collected and planted. In the resulting F₂ population, a plant containing the reduced apical dominant character was selected. This selected plant,

referred to as 3030-1-4, was then self-fertilized for two (2) generations.

The 3000 line was derived from a cross between *Eustoma* line 752 and “White Lisa”. Seed from this cross was collected and planted. An individual F₁ plant was selected and self-pollinated, and the resulting seed was collected and planted. In the resulting F₂ population, a plant containing the reduced apical dominant character was selected. This selected plant, referred to as 3000-2-3, was then self-fertilized for one (1) generation. Then the seed was massed and selfed for two (2) subsequent generations.

Eustoma hybrid 3213 contains the RAD allele and is a multiple branched dwarf *Eustoma* having a height of about 16 cm. The branching of this hybrid starts when the hybrid is in the plug stage. The flower is a campanulate type having a diameter of about 5.5 cm and is ivory in color (lighter than 00C0-C, 607C in the *Pantone Process Color Imaging Guide 1000*, 1992).

The leaves of this *Eustoma* are 5 x 3.5 cm (length x width) and are medium green.

Eustoma hybrid 3213 can be used in sexual crossings to introgress the RAD gene into the genetic backgrounds of other *Eustoma* plants having commercially desirable characteristics in order to create new plants exhibiting the RAD trait.

EXAMPLE 4: Comparison of Reduced Apical Dominance Hybrids to Commercial Hybrids

Five greenhouse plants of each hybrid listed in Table 1, below, were evaluated for basal branching and total branching. Plants were grown using standard greenhouse procedures and exposed to approximately 20 mol•m⁻²•day⁻¹ each day. The plants were evaluated after approximately three and one-half months growth for basal branching and after approximately four and one-half months growth for total branching. Commercial hybrids include the “Lisa” series and the “Florida” series both marketed by Pan American Seed, 622 Town Road, West Chicago, IL 60185. Basal branches are the branches arising

from the cotyledonary node below the first true leaves. Total branches include shoots arising from the basal branches and from the main stem above the first true leaves. Only branches longer than 2.5 cm were included in this count. Means were compared using the Least Significant Difference Test ($\alpha = .05$). Statistical analysis demonstrates that all hybrids having the Reduced Apical Dominance trait have significantly higher basal branching and total branching than the commercially available hybrids.

Table 1

Hybrid	Basal Branches	LSD= 1.02 $\alpha = .05$	Total Branches	LSD= 2.62 $\alpha = .05$
"Blue Lisa"	0 +/-0	a	1.0 +/- 0	a
"White Lisa"	0 +/-0	a	2.2 +/- 0.45	ab
"New Pink Lisa"	0 +/-0	a	1.8 +/- 0.84	ab
"Florida Blue"	0 +/- 0	a	1.6 +/- 0.89	ab
"Florida Sky Blue"	0.2 +/- 0.45	a	2.6 +/- 0.55	b
"Florida Pink"	0.2 +/- 0.45	a	1.8 +/-0.84	ab
3087	4.4 +/- 0.55	bcd	9.6 +/- 1.14	gh
3091	3.6 +/- 1.14	bc	5.2 +/- 0.84	c
3094	4.4 +/- 0.89	bcd	10.2 +/- 2.17	h
3210	3.8 +/- 0.45	bcd	6.8 +/- 1.3	de
3212	4.8 +/- 0.45	d	8.8 +/- 2.17	gh
3213	4.6 +/- 1.14	cd	9.0 +/- 1.22	gh
3214	4.8 +/- 1.1	d	7.2 +/- 1.1	ef
3211	3.4 +/- 1.67	b	8.4 +/- 1.52	fg
3215	4.2 +/- 1.3	bcd	5.4 +/- 1.14	cd

Figure 2 is a photograph comparing the RAD *Eustoma* hybrid 3087 (labeled as “A” in the photograph) with commercial hybrids “Blue Lisa” (labeled as “B” in the photograph) and “Florida Blue” (labeled as “C” in the photograph).

Figure 3 is a photograph comparing the RAD *Eustoma* hybrids 3214 (labeled as “A” in the photograph), 3213 (labeled as “B” in the photograph) and 3211 (labeled as “C” in the photograph) with commercial hybrid “White Lisa” (labeled as “D” in the photograph).

Figure 4 is a photograph comparing the RAD *Eustoma* hybrid 3215 (labeled as “A” in the photograph) with commercial hybrids “Pink Lisa”(labeled as “B” in the photograph) and “Florida Pink” (labeled as “C” in the photograph).

EXAMPLE 5: Demonstration of Reduced Apical Dominance Inheritance

Hybrids were generated from crosses having reduced apical dominance lines used as either the male or female. Plants were grown using standard greenhouse conditions and evaluated for the RAD trait after four (4) months. Evidence of RAD inheritance is provided in Table 2, below. Chi square analysis yields a value of 0.89 demonstrating a fit for a 3:1 segregation ratio.

Table 2

Hybrid	RAD Source	Total Plants	RAD Plants	% RAD
3109-1	3003-1-1-2-1	188	54	29
3109-2	3003-1-1-2-1	200	45	23
3128-1	1389-1-1-14-2	341	72	21

Deposit Information

Eustoma seeds of hybrid 3087 have been placed on deposit with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassus, Virginia, 20110-2209 under Deposit Accession Number 203392 on October 30, 1998. This deposit was made in compliance with the Budapest Treaty requirements that the duration of the deposit should be for thirty (30) years from the date of deposit or for five (5) years after the last request for the deposit at the depository or for the enforceable life of a U.S. Patent that matures from this application, whichever is longer. These *Eustoma* seeds will be replenished should it become non-viable at the depository.

The present invention is illustrated by way of the foregoing description and examples. The foregoing description is intended as a non-limiting illustration, since many variations will become apparent to those skilled in the art in view thereof. It is intended that all such variations within the scope and spirit of the appended claims be embraced thereby.

Changes can be made to the composition, operation and arrangement of the method of the present invention described herein without departing from the concept and scope of the invention as defined in the following claims.